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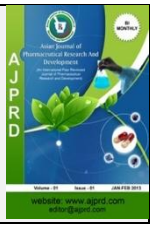

Open Access Research Article

Evaluation of the Larvicidal Activities of the Crude Root Extracts of *Ixora Coccinea* L (Rubiaceae) on *Aedes Aegypti* Larvae

Okwubie Lambert,*Ajogwu Amarachi Sophia

Department of Pharmacognosy and Phytotherapy, university of Port Harcourt, Nigeria

A B S T R A C T

In this study, the larvicidal activity of the crude root extract of *Ixora coccinea* was carried out. 400g of the plant material each was dried pulverized and extracted with sufficient quantity of water, methanol and acetone separately. The extracts were dried, and used for larvicidal assay. The assay was carried out According to WHO, 2005 guideline on larvicidal assay with some modifications. The activity was evaluated using six different concentrations of 0.5,1, 2, 3, 4, and 5mg/ml in volume of 100ml of water. It was run in triplicate with control for each concentration. 20 healthy larvae of 3rd instar was used for each of the triplicate and the control. The activity was monitored every 24 hours for 3 days. The number of death after every 24 hours was observed and recorded. The percentage mortality and the LC50 were calculated. Examination of the results showed that the acetone extract gave the highest mortality after 72 hours with 100% mortality and LC_{50} of 0.8mg/ml. The methanol extract gave 51.7% mortality with the highest concentration of 5mg/ml used and LC_{50} of 4.95mg/ml after 72 hours, while the aqueous extract gave 0% activity with the 5mg/ml highest concentration use after 72 hours. The result of this study showed that the acetone root extract of *Ixora coccinea* could further be worked on to isolated the active principle(s) with the larvicidal activity.

Keywords: *Ixora coccinea*, Root, Larvicidal

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***Address for Correspondence:**

Okwubie Lambert, Department of Pharmacognosy and Phytotherapy, university of Port Harcourt, Nigeria

INTRODUCTION

Serious human diseases are transmitted by mosquitoes, causing millions of death every year [(1)].The mosquito species *Aedes aegypti*is the species responsible for the transfer of various arbovirus diseases. The species is vector of viruses responsible for Yellow fever, Dengue fever, Chikungunya and Zika virus diseases etc. Only the female mosquito is capable of transmitting these viruses, since it feeds on human blood.

When there are good conditions particularly at high temperature and flooding, eggs of both Aedes aegypti and Aedes albopictus hatch within a few days into larvae. The larvae undergo thereafter four molts, which may take between 9 and 13 days, the male mosquitoes develop faster than the females and molt earlier into pupae. After a period of 2 days, the pupae develop further into adult mosquitoes [(2)]. These adult mosquitoes are the vector for the various diseases transmit by these species of mosquitoes. Since these diseases are transmitted by this mosquito, the control and regulation of these mosquitos is of paramount important in the prevention of these diseases. However, the dispersed and transient egg laying pattern of the female Aedes aegypti mosquito and its effective adaptation to the urban habitat, make the control of these mosquitoes challenging [(3)].

The plant *Ixora coccinea* L (Rubiaceae) is made up of leaves which are oblong, glossy and leathery. The leaves are also about 3-6 inch in length and have entire margins. The flowers come in different colours such as red, yellow, purple, orange, white, pink etc., and blossoms all through the year[(4)]. The plant has been shown to have some medicinal uses for instance Pharmacological studies suggest that the plant possesses anti-oxidative, antibacterial, gastroprotective, hepatoprotective , antidiarrheal,

antinociceptive, antimutagenic, antineoplastic and chemopreventive effects, thus lending scientific support to the plant's ethnomedicinal uses. [5].

The plant has also been shown to possess analgesic, anti-inflammatory, antipyretic effects [6] The leaf and flower of this plant have been shown to possess larvicidal activities against 4th instar of Anopheles and *Aedes aegypti* larvae [7].

The aim of this work is to evaluate the larvicidal activity of crude methanol, acetone and aqueous root extracts of *Ixora coccinea* on the mosquito species, *Aedes aegypti.*

Materials and methods

Materials

Some of the materials used in this work include analytical electronic weighing balance (model WT6002A, Thermostat water bath HH-6 (Techmel and Techmel USA), maceration jars, crucibles, Rotary evaporator, stainless steel spatula measuring cylinders, beaker, funnel, filter papers, desiccator etc.

Chemicals include; acetone, methanol, water, DMSO and solvents for phytochemical screenings etc.

Sample collection and identification.

The root sample of *Ixora coccinea* was collected from the university of Port Harcourt school gate at choba in rivers state Nigeria and it was identified by Dr. Sulieman of Department of Pharmacognosy and Phytotherapy, University of Port Harcourt and deposited at the same Department Herbarium.

Plant sample preparation

After the collection of sufficient quantity of the plant root sample, it was cut into smaller pieces and properly washed to remove sand and particles and dried under shade until well dried. It was then grinded with grinding machine. It was put in air tight container until time of extraction.

Extraction of the plant material using maceration method

400g of the sample was weighed and poured into the maceration jar, and 1.5 liter of analytical grade acetone was poured into the sample in the jar and covered. It was shaken or agitated for thorough mixing. The mixture was allowed to macerate for 24 hours with intermittent shaking for better extraction. It was then filtered. The process was repeated using the same marc for exhaustive extraction. The same process was used to extract with methanol and distilled water, using same quantity of fresh plant sample. The acetone and methanol extracts were concentrated using rotary evaporator and finally dried in a water bath at temperature of about 45^{O}C. The aqueous extract was however, dried only with water bath without been concentrated with rotary evaporator. All the dried extracts were stored in a desiccator till the time of use.

Phytochemical screening

The phytochemical screening was carried out according to the method by Harborne[8].

Preparation of stock solution

The stock solution of 10mg/ml was prepared for each of the extract by dissolving 6g of the extract in 600ml of water. Dissolution was facilitated in case of acetone extract with the use of about 5ml DMSO. From this stock solution serial dilution was done for the various concentrations used for the assay.

Larvicidal bioassay.

The larvicidal assay was carried out according to WHO guidelines for larvicidal assay with some modification [9].The Aedes aegypti egg for the assay was obtained from the National airborne viral research centre Enugu, Nigeria. It was hatch and the larvae grow in the department of Pharmacognosy and Phytotherapy of University of Port Harcourt. The assay was run with the larvae at the third instar stage of development. The assay was carried out in triplicate for each of the concentrations, along with a control for each of the concentration. The concentrations were 0.5, 1, 2, 3, 4, and 5mg/ml. 20 larvae was use for each container in a 100ml volume. The control was made of equal volume of water with the same number of larvae as is in those with the extract.

The activity was monitored every 24 hours for 3 days. This was done by counting the number of dead larvae every 24 hours for 72 hours. The results were taken, recorded and analyzed statistically.

Statistical Analysis

The statistical tools that were used in this study is the Ldp line software based on the standard method of probits by Finney The statistical tools that were used in this study is the Ldp line software based on the standard method of probits by Finney [11], to calculate LC 50 values of each extract after the various time of observations which are 24, 48 and 72 hours.

RESULTS

Table 1: Results showing the Percentage yield of the root extracts of *Ixora coccinea*extracts after extraction

Solvent	Quantity used (g)	Yield (g)	Percentage yield (%)
Acetone	400	26.06	6.52
Methanol	400	30.41	7.6
Aqueous	400	6.4	1.6

From the table one, methanol gave the highest yield of 7.6% followed by acetone with 6.52%, while the aqueous yield was the least with 1.6% only.

Result for phytochemical screening.

Examination of the results for the Phytochemical screening gave the presence of Alkaloids, Triterpenoids, Saponins, Phenols, Anthraquinones Carbohydrates and Cyanogenic glycosides.

Result for larvicidal assay.

Table 2: result for the larvicidal activity of crude acetone root extract of *Ixora coccinea* against Aedes aegypti larvae.

Conc. mg/ml	No. of larvae	Number of mortality After 24 hours					Number of mortality After 48 hours					Number of mortality After 72 hours				
		Replicate			Mean	C	Replicate			Mean	C	Replicate			Mean	C
		a	b	c			a	b	c		0	a	b	c		
0.5	20	1	1	0	0.7±0.8	0	6	7	2	5.0±2.2	0	7	8	5	6.67±2.16	0
1	20	3	0	3	2.0±1.9	0	9	6	13	9.3±2.9	0	12	7	14	11.0±2.9	0
2	20	3	3	6	4.0±1.4	0	12	12	14	12.7±1.6	0	13	15	16	14.7±2.16	0
3	20	3	4	4	3.7±0.8	0	15	17	16	16.0±0.8	0	17	17	19	17.7±1.6	0
4	20	8	10	9	9.0±0.8	0	15	18	17	16.7±2.2	0	20	20	20	20.0±0.0	0
5	20	15	14	18	15.7±1.7	0	20	19	19	19.3±0.8	0	20	20	20	20.0±0.0	0

Values for means are represented as mean of ± S.E.M (standard error of means).Note, C = control

Table 3: Result showing the percentage mortality and the LC_{50} for the larvicidal assay of acetone root extract of *Ixora coccinea* Aedes aegypti

Conc. mg/ml	% mortality after 24 hours		Extract LC_{50} After 24h	% mortality after 48 hours		Extract LC_{50} After 48h	% mortality after 48 hours		Extract LC_{50} After 72h
	Extract	control		Extract	control		Extract	control	
0.5	3.4±2.4	0		25.0±10.8	0		33.4±6.2	0	
1	10.0±7.1	0		46.7±14.3	0		55.0±14.7	0	
2	20.0±7.1	0	4.1 mg/ml	63.4±4.7	0	1.1 mg/ml	73.4±6.2	0	0.8 mg/ml
3	18.4±2.4	0		80.0±4.1	0		88.4±4.7	0	
4	45.0±4.1	0		83.4±6.2	0		100.0±0	0	
5	78.4±8.5	0		96.7±2.4	0		100.0±0	0	

Values for percentage mortality are represented as mean of ± S.E.M (standard error of means)

From the result on the table 2 above, the acetone crude root extract of *Ixora coccinea* gave strong larvicidal activity with LC_{50} of 4.05mg/ml after 24 hours and this decrease to 1.1mg/ml after 48 hours. It also further decrease to about 0.8mg/ml after 72 hours

Table 4: result for the larvicidal activity of crude methanol root extract of *Ixora coccinea* against Aedes aegypti larvae.

Conc. mg/ml	Number of larvae	Number of mortality After 24 hours					Number of mortality After 48 hours					Number of mortality fter 72 hours				
		Replicate			Mean	ctrl	Replicate			mean	ctrl	Replicate			Mean	ctrl
		a	c	d			a	b	c			a	B	C		
0.5	20	0	0	0	0.0±0.0	0	0	2	0	0.7±0.9	0	0	2	1	1.0±0.8	0
1	20	0	0	2	0.7±0.9	0	0	0	2	0.7±0.9	0	1	1	2	1.3±0.5	0
2	20	0	0	2	0.7±0.9	0	0	0	2	0.7±0.9	0	2	1	2	1.7±0.8	0
3	20	0	0	2	0.7±0.9	0	0	2	2	1.3±0.9	0	1	3	3	2.3±1.6	0
4	20	0	1	2	1.0±0.8	0	0	2	3	1.7±2.2	0	1	4	4	3.0±2.0	0
5	20	3	4	3	3.3±0.7	0	10	4	3	5.7±3.1	0	14	11	6	10.3±5.7	0

Values for mean are represented as mean of ± S.E.M (standard error of means)

Table 5: Result showing the percentage mortality and the LC_{50} for the larvicidal assay of methanol root extract of *Ixora coccinea* Aedes aegypti.

Conc. Mg/ml	% mortality after 24 hours		Extract LC_{50} After 24h	% mortality after 48 hours		Extract LC_{50} After 48h	% mortality after 48 hours		Extract LC_{50} After 72h
	Extract	control		Extract	control		Extract	Control	
0.5	0±0	0		3.4±3.3	0		5.0±4.1	0	
1	3.4±3.3	0		3.4±3.3	0		6.7±2.4	0	
2	3.4±3.3	0	49.7	3.4±3.3	0	27.3 mg/ml	8.4±2.4	0	5.0 mg/ml
3	3.4±3.3	0		6.7±4.7	0		11.7±4.7	0	
4	5.0±4.1	0		8.4±6.2	0		15±7.1	0	
5	16.7±2.4	0		28.4±15.5	0		51.7±16.5	0	

Values for percentage mortality are represented as mean of ± S.E.M (standard error of means)

From the result on the table 3 above it is very clear that the methanol crude root extract of *Ixora coccinea* gave mild larvicidal activity with highest LC_{50} of 4.95mg/ml after 72 hours. The activity is low when compare with that of acetone extract. Because of the zero activity of the 0.5mg/ml, the LC_{50} was analyzed from the 1mg/ml concentration.

Table 6: result for the larvicidal activity of crude aqueous root extract of *Ixora coccinea* against Aedes aegypti larvae.

Conc. mg/ml	Number of larvae	Number of mortality After 24 hours					Number of mortality After 48 hours					Number of mortality After 72 hours				
		Replicate			mean	Ctrl	Replicate			mean	ctrl	Replicate			Mean	ctrl
		a	b	c			A	b	c			A	b	C		
0.5	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0
1	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0
2	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0
3	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0
4	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0
5	20	0	0	0	0±0	0	0	0	0	0±0	0	0	0	0	0±0	0

Values for means are represented as mean of ± S.E.M (standard error of means)

Table 7: Result showing the percentage mortality and the LC_{50} for the larvicidal assay of aqueous root extract of *Ixora coccinea* Aedes aegypti

Conc. Mg/ml	% mortality after 24 hours		extract LC_{50} After 24h	% mortality after 48 hours		extract LC_{50} After 48h	% mortality after 48 hours		Extract LC_{50} After 72h
	Extract	Control		Extract	Control		extract	control	
0.5	0±0	0		0±0	0		0±0	0	
1	0±0	0		0±0	0		0±0	0	
2	0±0	0	0 mg/ml	0±0	0	0 mg/ml	0±0	0	0 mg/ml
3	0±0	0		0±0	0		0±0	0	
4	0±0	0		0±0	0		0±0	0	
5	0±0	0		0±0	0		0±0	0	

Values for percentage mortality are represented as mean of ± S.E.M (standard error of means)

From the result in table 7 above, it is very clear that the aqueous extract showed zero larvicidal activity with LC_{50} of 0 mg/ml

DISCUSSION

In the fight against troublesome insects, plant kingdom has been shown to be a reliable source of biochemical for insecticides. For instance, Roark [12] describe about 1,200 plant species having potential insecticidal value, also Sukumar[13] has listed and discussed 344 plant species with toxic effect on mosquitoes. The need to find biochemical means of fighting mosquito species which is the vector to many diseases causing microbes cannot be overemphasis. Biochemical means are emphasized because some of the synthetic chemical has strong disadvantages such as development of resistance by the insects, not been friendly to the eco-system as they may be toxic to both animals and man. In regard of the effort to find biochemical agents with strong insecticides activity, this work evaluates the larvicidal activity of crude acetone, methanol and aqueous extracts of *Ixora coccinea* root against *Aedes aegypti*

The result of the extraction of the root extracts with methanol, acetone and water gave different percentage yield. Methanol gave the highest yield of 7.6%, this was followed by acetone with 6.52% and then water with the list amount of 1.6%. the different percentage yield may be due to difference in the polarity index of the solvents

The result on the Phytochemical screening of the root of the plant *Ixora coccinea* showed the presence of Alkaloids, Triterpenoids, Saponins, Phenols, Anthraquinones Carbohydrates and Cyanogenic glycosides. The larvicidal activity of the crude root extract of *Ixora coccinea* may be due to the action of one or more of these Phytochemical.

Examination of the results on larvicidal activity showed that the acetone extract gave the highest activity after 72 hours with LC50 of 0.8mg/ml with 100% mortality, while methanol extract gave LC_{50} of 4.95mg/ml after 72 hours with maximum of 51.7% mortality. On the other hand the aqueous extract gave LC_{50} of 0 mg/ml and 0% mortality.

Suryawanshi et al., [7] have shown that the leaf and flower extracts of *Ixora coccinea* possess larvicidal activities against 4th instars anopheles and *Aedes aegypti* mosquito. Thus motivating the evaluation of the root extracts for larvicidal activities as done in this work and the result above gave strong activity particularly for the acetone extract.

It is important to note from the results that the activities of these extracts that were active were time and concentration dependent, that is as the time of exposure and concentration increase the activity also increase. This has also been

observed in other work on larvicidal assay as shown by Okwubie and John [14], Nwabor et al, [15] and Ubulom et al, [16]even though their work was not on the same plant species with this work. However, the general observation is that the larvicidal activities are concentration and time dependent.

CONCLUSION

The crude acetone root extract of *Ixora coccinea* has strong larvicidal activity against *Aedes aegypt* and effort could be made to isolate and characterize the active principle responsible for the larvicidal activity, which could be helpful in the fight against the scourge of *Aedes Aegypti* mosquito borne diseases.

REFERENCE

1. Kamaraji C, Bagavan A, Elango G, Abduz Zahir A, Rajakumar G, Marimuthu S, Santhoshkumar T, Abdul Rahuman A. larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*, Ind. J. Med. Res. 2011; 134(1):101-106
2. Olivia Wesula Leande, Vincent Obanda, Anders Lindstrom, Clas Ahlm Magnus Evander Jones Naslund and Goran Buct; Globe-Trotting *Aedes aegypti* and *Aedes albopictus;* Risk Factors for Arbovirus pandemic; Vector-Borne and Zoonotic Disease; 2020; 20(2):71-81.
3. Muktar, Y, Tamerat N, Shewafera A, *Aedes aegypt* as a vector of Flavivirus J. trop. dis. 2016 4(5):223.
4. Whistler W.A., Tropical ornamentals, a guide. 1st ed. Timber press, Portland, Oregon, USA: 2000. p. 542
5. Shrinath Baliga and Kurian John Poruthukaren. Ixora cocinnea linn Traditional uses, Phytochemistry and Pharmacology. Chinese Journal of Integrative Medicine 2012; 18(1)72-79.
6. M.S Ali Adnan, M.M. Al-Amin, Mir M.N Uddin, M. Shohel, R. Bhattacharjee, J.M.A. Hannan. Analgesic, anti-inflammatory and antipyretic effects of *Ixora coccinea* Journal of Basic and Clinical Physiology and Pharmacology, 2014; 25(4):1-6
7. Rahul suryawanshi, Chandrashekhar Patil, Hemant Borase, Chandrakant Narkhede & Satish Patil, Screening of Rubiaceae and Apocynaceae extracts for mosquito larvicidal potential. Natural product research 2015; 29(4)353 -358
8. Harborne, J.B. Phytochemical Methods.2nd ed. Chapman and Hall, London. 1984, p. 166-226.
9. WHO, Guidelines for Laboratory and Field Testing of Mosquito larvicides. World Health Organization, Geneva 2005, p. 1-39
10. Finney, D.J. Probit analysis.3rd ed. Cambridge University Press, Cambridge; 1971
11. Roark, R.C; Some promising insecticidal plants. Econ Bot, 1947; 1:437-45,
12. Sukumar k, Perich M.J, Boobar L R. Botanical derivatives in mosquito control: a review. Journal of the American Mosquito Control Association, 1991; 7(2):2010-237.
13. Okwubie Lambert and Dan John. Evaluation of Larvicidal Activities of Stem Bark and Seed Extracts of *Picrilima nitida* (Stapf.) T. Durand and H. Durand (Apocynaceae) on *Aedes Aegypti* Larvae. International Journal of Pharmacy and Biological Sciences, 2017; 7(3):65-70
14. Nwabor O.F, Dibua U.M, Nnamonu E. I, Odiachi Osita, Dickson I. Dickson, Okoro O.J. Pulp Extracts of *Picralima nitida*: A Larvicidal Agent in Malaria Vector Control. Journal of Biology, Agriculture and Healthcare, 2014; 4(8):69-73.
15. Ubulom PME, Imandeh NG, Udobi CE, IIya I. Larvicidal and antifungal properties of *Picralima nitida* (Apocynaceae) leaf extracts. Eur J Med Plants, 2012; 2(2):132-139.